



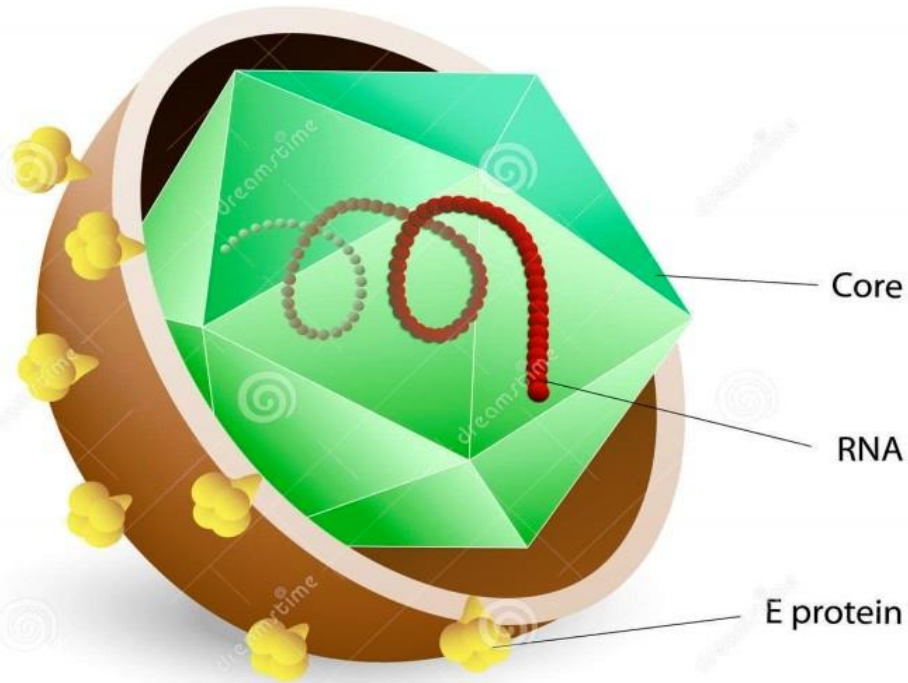
Global Hepatitis Outbreak & Surveillance Technology (GHOST) in Michigan

Kevin M. Rodeman
MDHHS Bureau of Laboratories

GHOST

- ▶ GHOST is a new web-based system that harnesses the power of novel bioinformatics technology and automatically performs a comprehensive analysis at the click of a button. Once a user inputs an HCV sample's unique fingerprint, GHOST does a quality control check, identifies transmission links between other virus samples, and automatically creates a simple graph that shows plainly which cases are linked by transmission.
- ▶ Goal is to enable state public health laboratories to conduct independent and efficient outbreak investigation and molecular surveillance of Hepatitis C virus.

Hepatitis C virus



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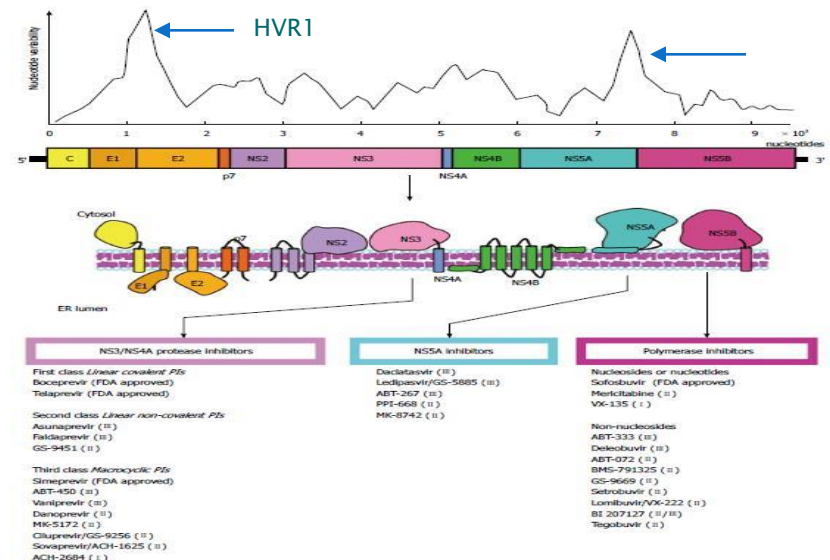


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HCV Heterogeneity

Genetic heterogeneity is distributed unevenly along HCV genome. Heterogeneous regions are most useful for molecular surveillance (see arrows).

HVR1 is used to sample intra-host HCV variants from infected individuals for outbreak investigation.



HCV Quasispecies

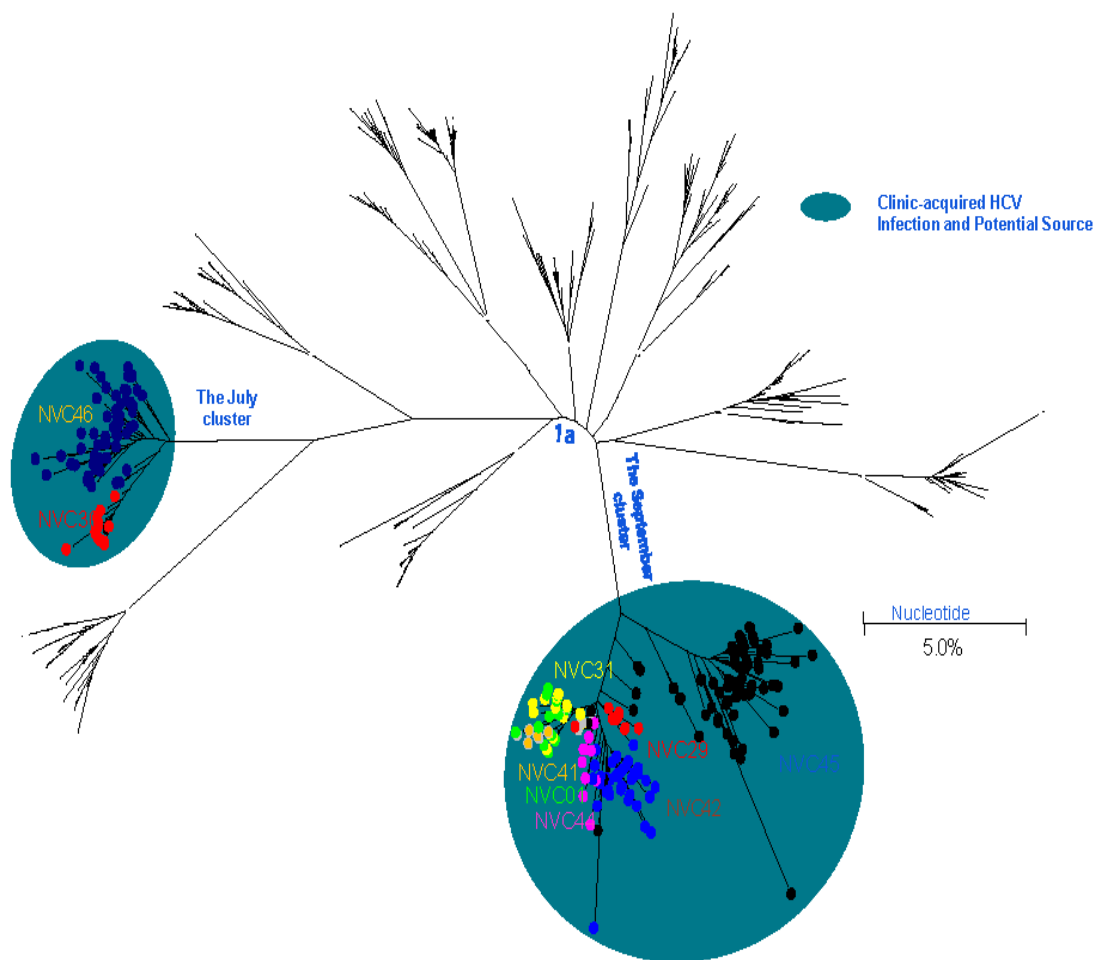
In infected hosts, HCV exists as a large population of closely related but distinct variants.

This occurs through reinfection or mutations over time in host.

How to Track Outbreaks?

- ▶ Sanger Sequencing of Each Infected Case to Yield a Consensus Sequence
- ▶ Consensus Sequences are not Satisfactory for accurate ID of viral strains–Quasispecies
- ▶ End–Point Limiting Dilution Technique
- ▶ Next Generation Sequencing
- ▶ Create a Phylogenetic Tree of Cases

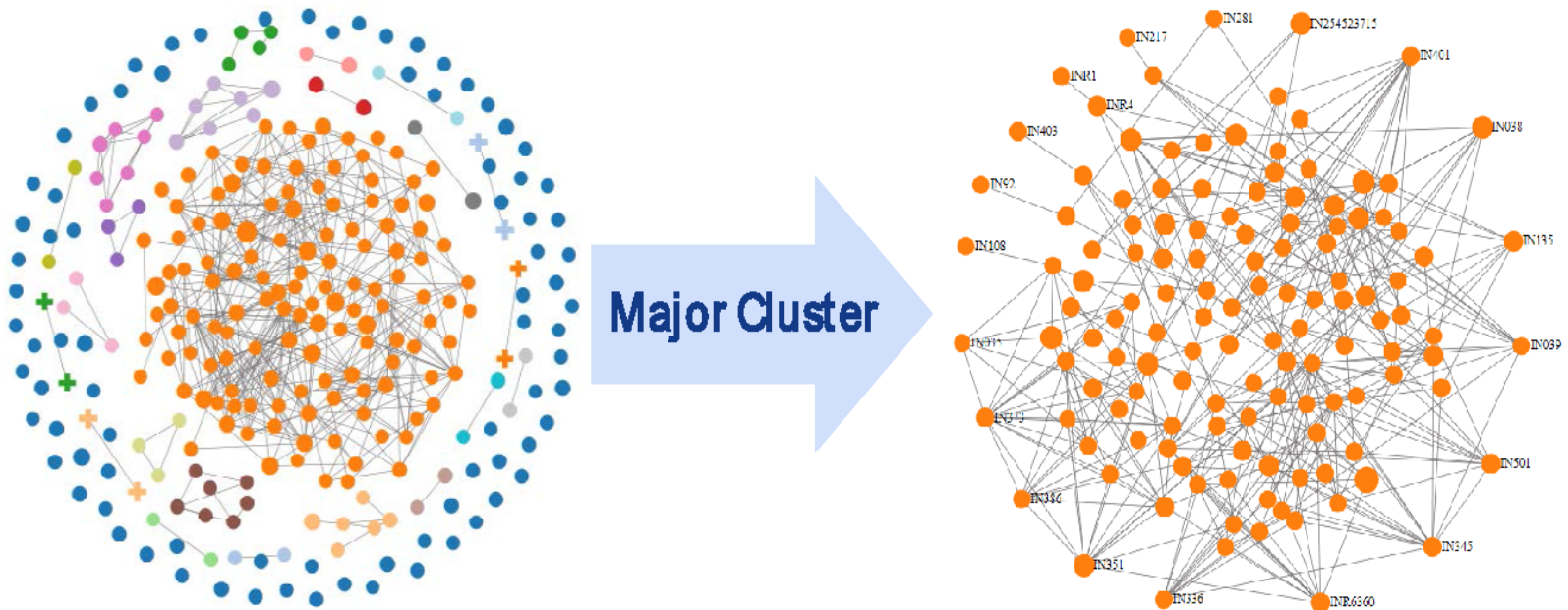
Traditional Phylogenetic Analysis



Next Generation Sequencing

- ▶ NGS is used to sample intra-host HVR1 variants from infected individuals.
- ▶ Single amplicon sequencing about 320bp.
- ▶ 10^5 – 10^7 sequences from a single run.
- ▶ Multiplexing: sequencing of dozens of HCV strains in a single test.
- ▶ Significant cost reductions per sequenced strain.

GHOST Analysis

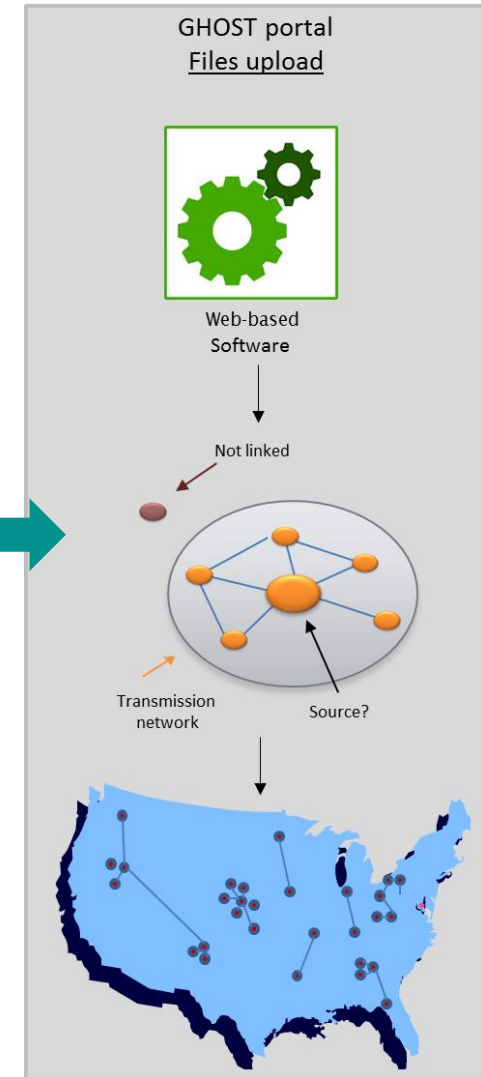
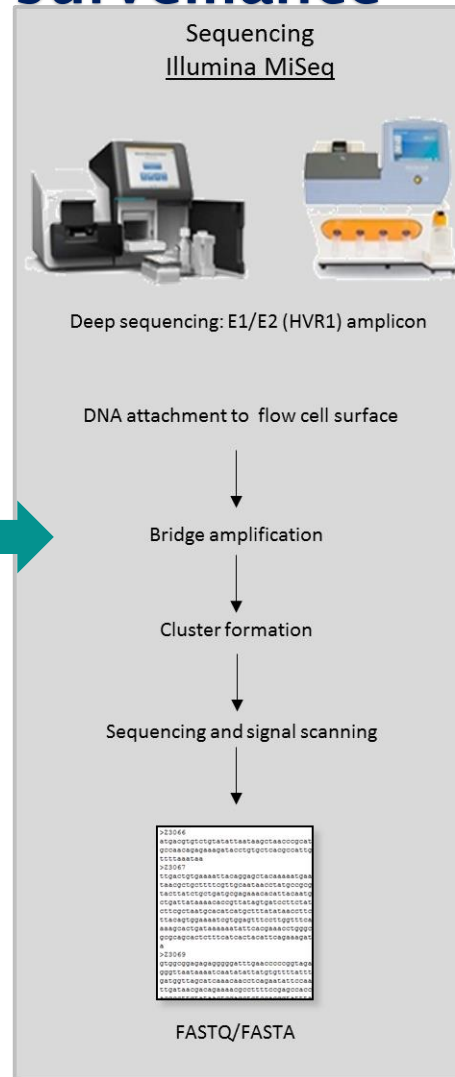
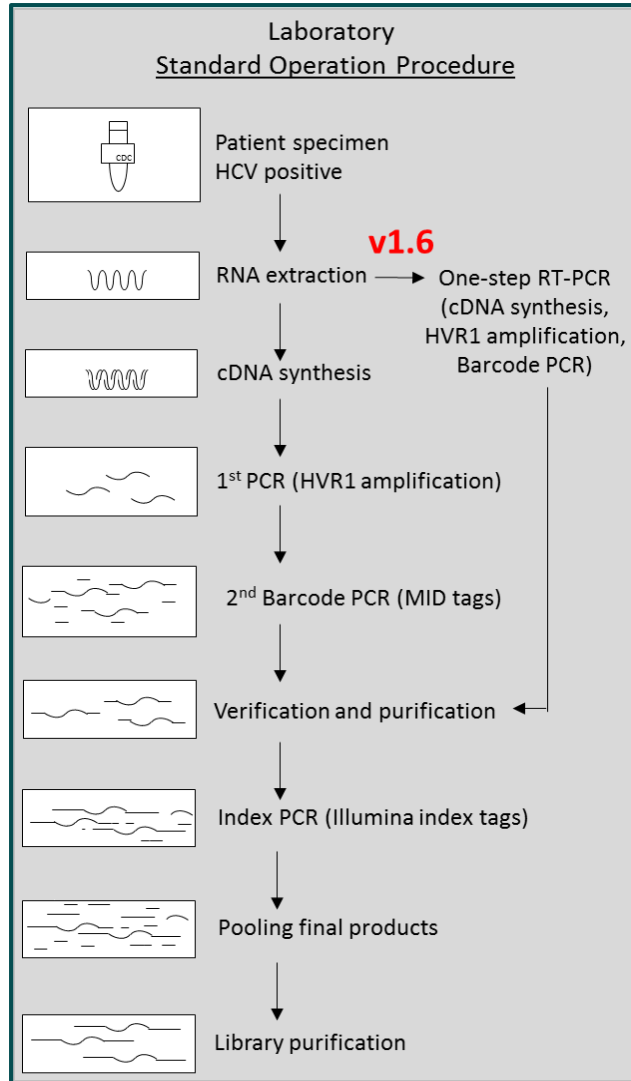


GHOST was an essential part of the “The Indiana Injection Drug Use-Associated HIV/HCV Outbreak Response Team”, which was selected as the winner of the 2015 CDC/ATSDR Honor Award for Excellence in Emergency Response – Domestic.

HCV Outbreaks Associated with the Following:

- ▶ Unsafe Injection Practices
- ▶ Drug Diversion
- ▶ Exposure to Blood and Blood Products
- ▶ MSM

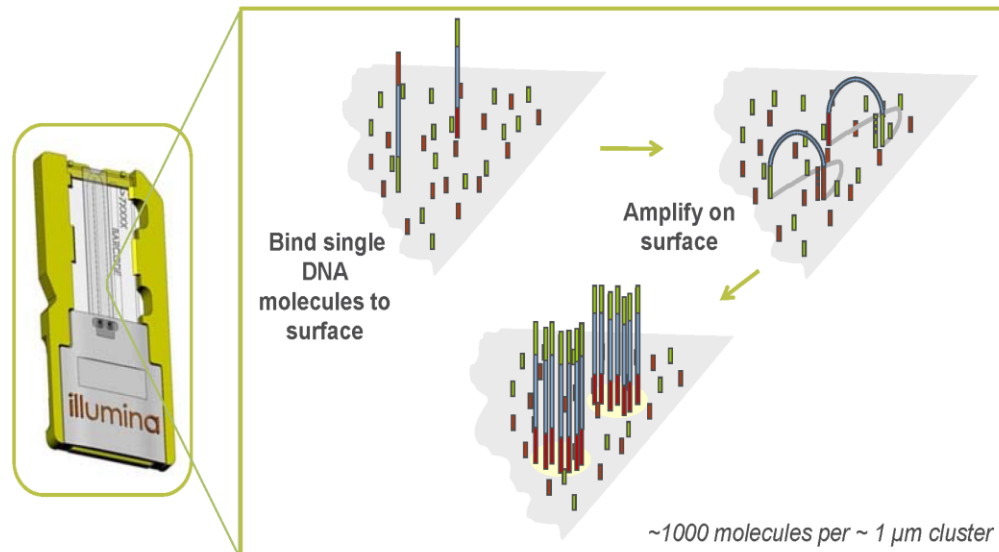
GHOST Workflow: HCV outbreak detection & molecular surveillance






MISeq Flow Cell

Cluster Generation Overview



[Skip Details](#)



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Alpha 0.6.6

GHOST

User: Rodeman, Kevin Global Health Outbreak and Surveillance Technology (GHOST) - Version 0.6.6 Role: User

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Task ID	Name	Description	Task Type	Country	State	Status	Date	Data Sets	Owner
402	Kevin		Analysis	United States	MI	Complete	04/03/2017	CLEAN(4) ANALYSIS(Rodeman, Kevin
398	Michigan Specimens GHOST 3/31/2017	First set of 15	Analysis	United States	MI	Complete	03/31/2017	CLEAN(14) ANALYSIS	Rodeman, Kevin
391	Michigan GHOST Specimens 03/31/2017	First 15 specimens	Quality Control	United States	MI	Warning	03/31/2017	RAW(32) CLEAN(14)	Rodeman, Kevin
388	Michigan GHOST Panel II 03272017	Third Run different MISec	Analysis	United States	MI	Complete	03/27/2017	CLEAN(11) ANALYSIS	Rodeman, Kevin
387	Michigan GHOST Panel II 03272017		Quality Control	United States	MI	Warning	03/27/2017	RAW(48) CLEAN(11)	Rodeman, Kevin
383	GHOST Panel II-MI		Analysis	United States	MI	Complete	03/13/2017	CLEAN(8) ANALYSIS(Rodeman, Kevin
381	Michigan GHOST Panel II	verification panel	Quality Control	United States	MI	Warning	03/13/2017	RAW(48) CLEAN(8)	Rodeman, Kevin
327	Michigan-CDC GHOST Panel-02102017	on site procedure verifi	Analysis	United States	MI	Complete	02/10/2017	CLEAN(7) ANALYSIS(Rodeman, Kevin
324	Michigan GHOST Panel	Michigan CDC Verification	Quality Control	United States	MI	Warning	02/09/2017	RAW(16) CLEAN(7)	Rodeman, Kevin

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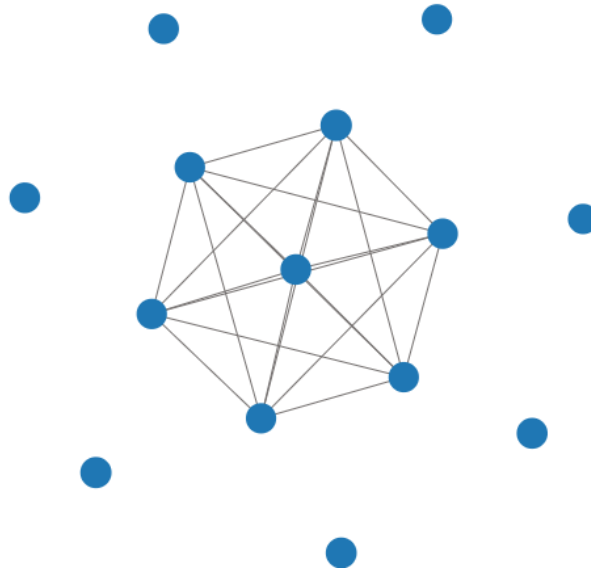
Global Health Outbreak and Surveillance Technology (GHOST) - Version 0.6.6 (Build 001)
For technical issues, please send e-mail to nchhstpinformatics@cdc.gov.

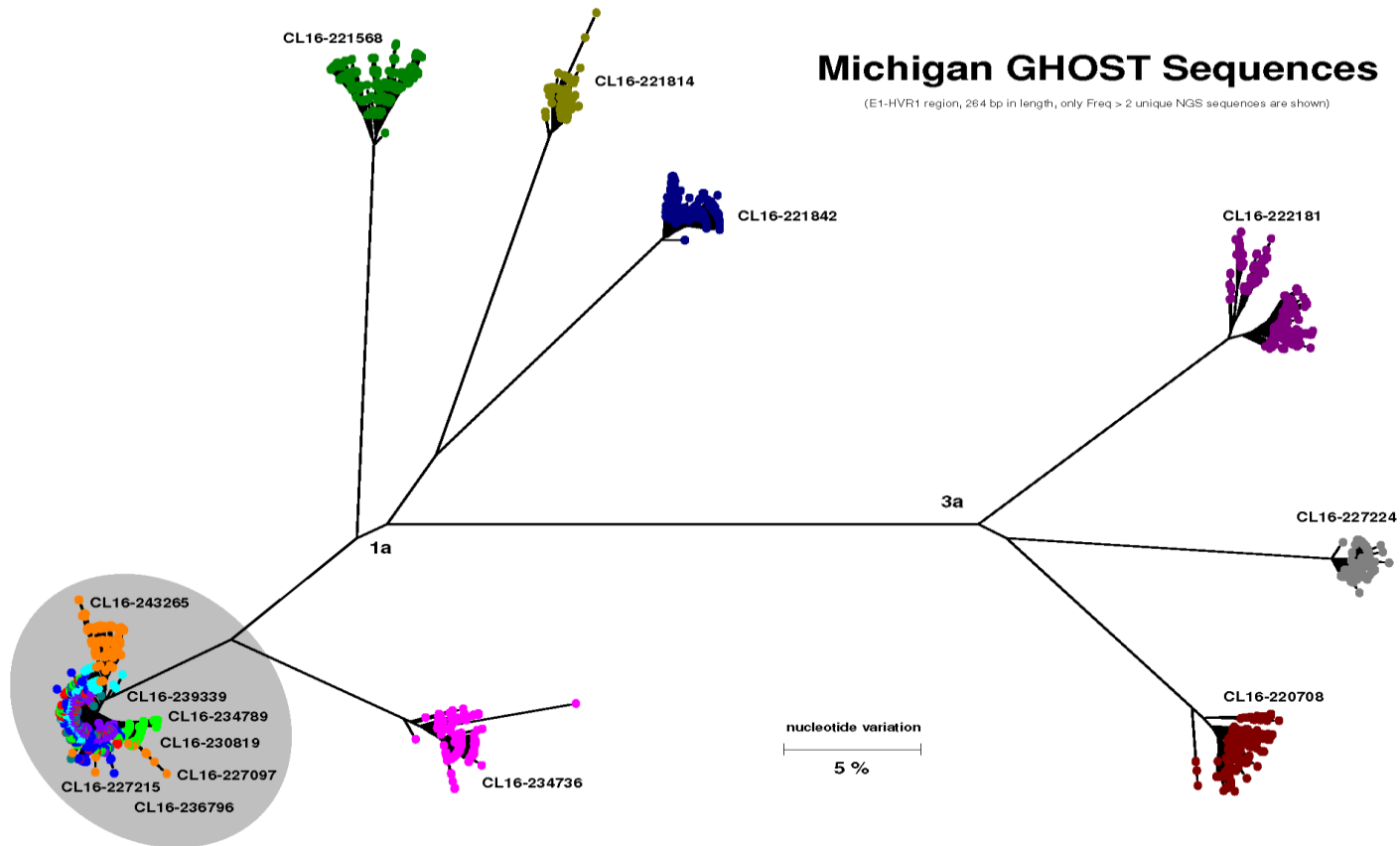
Data from GHOST

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CL16-230789_S8_L001_R1_001.fastq	CL16-239339_S4_L001_R1_001.fastq	0	1a	1a
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CL16-230789_S8_L001_R1_001.fastq	CL16-227215_S2_L001_R1_001.fastq	0	1a	1a
CL16-230789_S8_L001_R1_001.fastq	CL16-236796_S5_L001_R1_001.fastq	0	1a	1a
CL16-230789_S8_L001_R1_001.fastq	CL16-230819_S7_L001_R1_001.fastq	0	1a	1a
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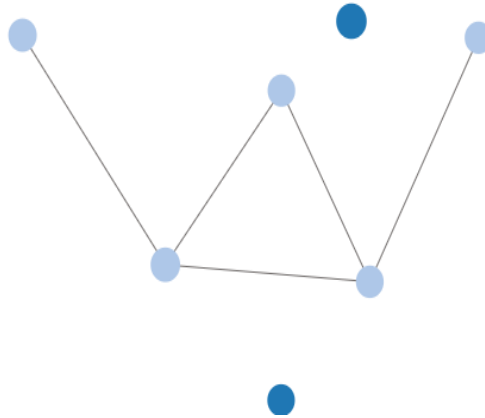
HCV Specimens Analyzed by GHOST



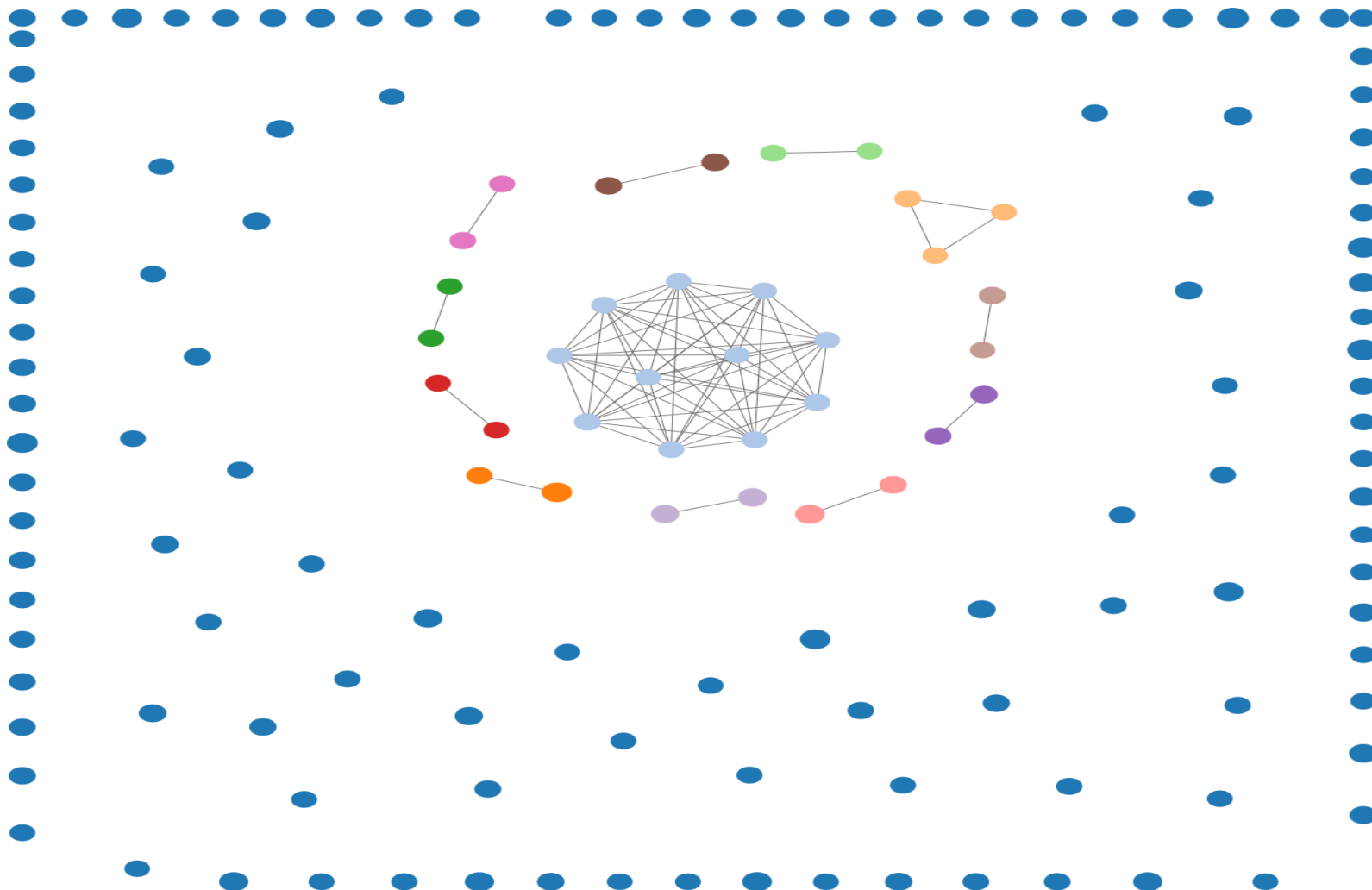
Phylogenetic Tree MI GHOST Sequences



Unable to Determine Direction of Infection



GHOST 1 to 11



GHOST Price per Specimen (16)

Item	Manufacturer	Catalog No.	Price (\$)	Price/ Specimen (\$)
easyMAG	BioMérieux			\$ 20.00
OneStep RT-PCR Kit	QIAGEN	210212	\$ 421.60	\$ 6.60
RT-PCR Primers (F/R)	Sigma			\$ 0.45
PerfeCTa SYBR Green FastMix	QUANTA	95072-012	\$ 839.98	\$ 0.66
Index PCR primers (F/R)	Sigma			\$ 0.02
Strip tube with attached cap	VWR	20170-004	\$ 47.02	\$ 0.75
Bio-Analyzer 4200 TapeStation	Agilent	D1000 Screen Tapes	\$ 215.00	\$ 4.60
		D1000 Reagents	\$ 92.10	
		Consumables	\$ 26.90	
AMPure XP Beads	Beckman Coulter	A63880	\$ 416.00	\$ 2.70
Storage Bulk Plate	Fisher Scientific	AB-0859	\$ 211.00	\$ 4.22
PhiX v3	Illumina	FC-1110-3001	\$ 160.00	\$ 35.55
MiSeq Reagent Nano Kit v2 500 Cycles	Illumina	MS-103-1003	\$ 360.00	\$ 22.50
Consumables*				\$ 5.00
Total				\$ 103.05
4200 TapeStation PM Contract	Agilent	TS0571	\$ 4,140.00	\$ 21.60
MiSeq Service Contract	Illumina	SV-420-1003	\$ 15,470.75	\$ 80.60
Grand Total				\$ 205.25

* pipet tips, 100% ethanol, RNF water, 1 N Sodium hydroxide, molecular grade bleach, 1.7 and 2.0mL screw cap tubes.

Moving Forward

- ▶ Based on the data from GHOST pilot project, write up a final procedure.
- ▶ A GHOST Run a month, depending on workload.
- ▶ Reaching out to other facilities for specimens of interest to test.